

United States Department of Agriculture  
Center for Veterinary Biologics  
Testing Protocol

SAM 512

Supplemental Assay Method for the Determination  
Of Formaldehyde in Veterinary Biologics  
(Ferric Chloride Test)

Date: April 4, 2005  
Number: TCSAM0512.02  
Supersedes: TCSAM0512.01, Dated July 7, 2003  
Standard Requirement: 9 CFR 113.100 (f), 9 CFR 113.200 (f)  
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Supplemental Assay Method for the Determination of Formaldehyde  
in Veterinary Biologics (Ferric Chloride Test)

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## 1. Introduction

This Supplemental Assay Method (SAM) describes how total formaldehyde is determined based on the reaction of formaldehyde with Methylbenzothiazolone hydrazone hydrochloride (MBTH). The method involves (a) the combination of MBTH and formaldehyde to give one product, (b) the oxidation of excess MBTH to give another product and (c) the combination of these two to give a blue chromophore which is measured at 628 nm<sup>2</sup>.

## 2. Materials

### 2.1 Reagents

**2.1.1** Ferric chloride-sulphamic acid reagent. A solution containing 10 g/L of ferric chloride and 16 g/L of sulphamic acid.

**2.1.2** Methylbenzothiazolone hydrazone hydrochloride reagent. (MW 233.7). [CAS 149022-15-1]. 3-Methylbenzothiazol-2(3H)one hydrazone hydrochloride monohydrate. An almost white or yellowish crystalline powder. mp: about 270°C. A solution containing 0.5 g/L L. (**WARNING: This solution is not stable and should be prepared fresh daily.**)

**2.1.3.** Suitability for determination of aldehydes. To 2 ml of aldehyde-free methanol add 60 µl of a 1 g/L solution of propionaldehyde in aldehyde-free methanol and 5 ml of a 4 g/L solution of methylbenzothiazolone hydrazone hydrochloride. Mix, allow to stand for 30 minutes. Prepare a blank omitting the propionaldehyde solution. Add 25.0 ml of a 2 g/L solution of ferric chloride to the test solution and to the blank, dilute to 100.0 ml with acetone R and mix. Measure absorbance of the test solution on a spectrophotometer at 660 nm in a 1 cm cell using the blank as compensation liquid. The absorbance of the test solution must be greater than or equal to 0.62 absorbance units.

**2.1.4.** Formaldehyde solution, containing not less than 34.5 percent w/v and not more than 38.0 percent w/v of formaldehyde (CH<sub>2</sub>O)

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- 2.1.5. Isopropyl myristate, analytical grade
- 2.1.6. Hydrochloric acid (1 M), analytical grade
- 2.1.7. Chloroform, analytical grade
- 2.1.8. Sodium chloride (9 g/L and 100 g/L aqueous solutions), analytical grade
- 2.1.9. Polysorbate 20, analytical grade

**3. Preparation for the test**

**3.1 Personnel qualifications/training**

No specific training is required. Individual should have working knowledge of laboratory equipment listed in **Section 2**.

**3.2 Preparation of Standards**

Prepare formaldehyde standards of 0.25, 0.50, 1.00 and 2.00 g/L by diluting formaldehyde solution (1.3) with water in suitable volumetric flasks.

**3.3 Preparation of vaccines containing oil emulsion**

If vaccine to be examined is an oil emulsion, the emulsion should be broken by a suitable method. The formaldehyde concentration in the aqueous phase should be measured. The following separation techniques have been shown to be appropriate.

**3.3.1** Add 1.00 ml of vaccine to 1.0 ml of isopropyl myristate and mix. To the mixture, add 1.3 ml of 1 M hydrochloric acid, 2.0 ml of chloroform and 2.7 ml of 9 g/L sodium chloride. Mix thoroughly. Centrifuge at 15,000 g for 60 minutes. Transfer the aqueous phase to a 10-ml volumetric flask and dilute to volume with water. Use the diluted aqueous phase for the test for formaldehyde. If this procedure described fails to separate the aqueous phase, add 100 g/L of polysorbate 20 to the sodium chloride solution and repeat the procedure, but centrifuge at 22,500 g.

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**3.3.2** Add 1.00 ml of vaccine to 1.0 ml of a 100 g/L solution of sodium chloride and mix. Centrifuge at 1000 g for 15 minutes. Transfer the aqueous phase to a 10-ml volumetric flask and dilute to volume with water. Use the diluted aqueous phase for the test for formaldehyde.

**3.3.3** Add 1.00 ml of vaccine to 2.0 ml of a 100 g/L solution of sodium chloride and 3.0 ml of chloroform and mix. Centrifuge at 1000 g for 5 minutes. Transfer the aqueous phase to a 10-ml volumetric flask and dilute to volume with water. Use the diluted aqueous phase for the test for formaldehyde.

**NOTE:** Volumes used for breaking emulsions are for the purpose of illustration. Volumes may differ subject to proportional adjustment of the volumes of other reagents used in the extraction process.

**4. Performance of the Test**

**4.1** To 0.50 ml of a 1 in 200 dilution of the vaccine to be examined (if emulsion, use 0.50 ml of a 1 in 20 dilution of the diluted aqueous phase), and to 0.50 ml of 1 in 200 dilution of each of the formaldehyde standards, add 5.0 ml of the methylbenzothiazolone hydrazone hydrochloride reagent. Close the tubes, shake, and allow to stand for 60 minutes.

**4.2** Add 1 ml of ferric chloride-sulphamic acid reagent and allow to stand for 15 minutes.

**4.3** Measure absorbance of vaccines and standards on a spectrophotometer at the maximum at 628 nm in a 1 cm cell, using the reagent blank as compensation liquid.

**5. Calculations and Interpretation**

Calculate total formaldehyde concentration (g/L) from the standard curve using linear regression (acceptable correlation coefficient [r] equal to or greater than 0.97).

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**6. Report of the test results**

Test results are reported following the current version of TCSOP0001.

**7. References**

**7.1** Code of Federal Regulations, Title 9, Parts 113.100(f) and 113.200(f), U.S. Government Printing Office, Washington, DC, 2004.

**8. Summary of revisions**

This document was revised to clarify the practices currently in use at the Center for Veterinary Biologics and to provide additional detail. While no significant changes were made that impact the outcome of the test, the name of the contact person has changed.